

CLAIMS

1. A recombinant allergen, characterised in that it is a mutant of a naturally occurring allergen, wherein the mutant allergen has at least four primary mutations, which each reduce the specific IgE binding capability of the mutated allergen as compared to the IgE binding capability of the said naturally occurring allergen, wherein each primary mutation is a substitution of one surface-exposed amino acid residue with another residue, which does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic species from which said naturally occurring allergen originates, wherein each primary mutation is spaced from each other primary mutation by at least 15 Å, and wherein the primary mutations are placed in such a manner that at least one circular surface region with a area of 800 Å² comprises no mutation.
2. A recombinant allergen according to claim 1, wherein the primary mutations are spaced 20 Å, preferably 25 Å and most preferably 30 Å.
3. A recombinant allergen according to claim 1 or 2 comprising a number of secondary mutations, which each reduce the specific IgE binding capability of the mutated allergen as compared to the binding capability of the said naturally occurring allergen, wherein each secondary mutation is a substitution of one surface-exposed amino acid residue with another residue, which does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic species from which said naturally occurring allergen originates, wherein the secondary mutations are placed outside the said circular region.

4. A recombinant allergen according to any of claims 1-3, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen has a solvent accessibility of above 20 %, preferably above 30 %, more preferably above 40 % and most preferably above 50 %.
5. A recombinant allergen according to any of claims 1-4, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen is conserved with more than 70 %, preferably 80 % and most preferably 90 % identity in all known homologous proteins within the species from which said naturally occurring allergen originates.
6. A recombinant allergen according to any of claims 1-5, which essentially has the same α -carbon backbone tertiary structure as said naturally occurring allergen.
7. A recombinant allergen according to any of claims 1-6, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic genus, preferably the subfamily, more preferably the family, more preferably the superfamily, more preferably the legion, more preferably the suborder and most preferably the order from which said naturally occurring allergen originates.
8. A recombinant allergen according to any of claims 1-7, characterised in that the specific IgE binding to the mutated allergen is reduced by at least 5%, preferably at least 10%.
9. A recombinant allergen according to claim 6, characterised in that when comparing the α -carbon

backbone tertiary structures of the mutant and the naturally occurring allergen molecules, the average root mean square deviation of the atomic coordinates is below 2Å.

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10. A recombinant allergen according to any of claim 1-9, characterised in that said circular surface region comprises atoms of 15-25 amino acid residues.

10 11. A recombinant allergen according to any one of claims 1-10, characterised in that the surface-exposed amino acid residues are ranked with respect to solvent accessibility, and that one or more amino acids among the more solvent accessible ones are substituted.

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12. A recombinant allergen according to any one of claims 1-11, characterised in that the surface-exposed amino acid residues are ranked with respect to degree of conservation in all known homologous proteins within the species from which said naturally occurring allergen originates, and that one or more amino acids among the more conserved ones are substituted.

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13. A recombinant allergen according to any of claims 1-12, wherein the mutant allergen is a non-naturally occurring allergen.

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14. A recombinant allergen according to any of claims 1-13 comprising from 5 to 20, preferably from 6 to 15, more preferably from 7 to 12, and most preferably from 8 to 10 primary mutations.

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15. A recombinant allergen according to any one of claims 1-14 characterised in that the mutant allergen comprises from 1 to 4 secondary mutations per primary mutation.

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16. A recombinant allergen according to any one of claims 1-15, characterised in that one or more of the substitutions is carried out by site-directed mutagenesis.

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17. A recombinant allergen according to any one of claims 1-16, characterised in that one or more of the substitutions is carried out by DNA shuffling.

10 18. A recombinant allergen according to any one of claims 1-17 characterised in that it is a mutant of an inhalation allergen.

15 19. A recombinant allergen according to claim 18, characterised in that it is a mutant of a pollen allergen.

20 20. A recombinant allergen according to claim 19 characterised in that it is a mutant of a pollen allergen originating from the taxonomic order of *Fagales*, *Oleales* or *Pinales*.

21. A recombinant allergen according to claim 20, characterised in that it is a mutant of *Bet v 1*.

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22. A recombinant allergen according to claim 21, characterised in that one or more of the substitutions is selected from the group consisting of V2, D72, E87, K-129, E-60, N-47, K-65, P-108, N-159, D-93, K-123, K-32,
30 D-125, R-145, D-109, E-127, Q-36, E-131, L-152, E-6, E-96, D-156, P-63, H-76, E-8, K-134, E-45, T-10, V-12, K-20, S-155, H-126, P-50, N-78, K-119, V-2, L-24, E-42, N-4, A-153, I-44, E-138, G-61, A-130, R-70, N-28, P-35, S-149, K-103, Y-150, H-154, N-43, A-106, K-115, P-14, Y-5,
35 K-137, E-141, E-87 and E-73.

23. A recombinant allergen according to claim 19, characterised in that it is a mutant of a pollen allergen originating from the taxonomic order of *Poales*.
- 5 24. A recombinant allergen according to claim 19, characterised in that it is a mutant of a pollen allergen originating from the taxonomic order of *Asterales* or *Urticales*.
- 10 25. A recombinant allergen according to claim 18, characterised in that it is a mutant of a house dust mite allergen.
- 15 26. A recombinant allergen according to claim 25, characterised in that it is a mutant of a mite allergen originating from *Dermatophagoides*.
- 20 27. A recombinant allergen according to claim 18, characterised in that it is a mutant of a cockroach allergen.
- 25 28. A recombinant allergen according to claim 18, characterised in that it is a mutant of an animal allergen.
29. A recombinant allergen according to claim 28, characterised in that it is a mutant of an animal allergen originating from cat, dog or horse.
- 30 30. A recombinant allergen according to any one of claims 1-17 characterised in that it is a mutant of a venom allergen.
- 35 31. A recombinant allergen according to claim 30, characterised in that it is a mutant of a venom allergen originating from the taxonomic order of *Hymenoptera*.

32. A recombinant allergen according to claim 31, characterised in that is a mutant of a venom allergen from the taxonomic order of Vespidae, Apidae and Formicoidae.

33. A recombinant allergen according to any one of claims 30-32 characterised in that it is a mutant of Ves v 5.

34. A recombinant allergen according to claim 33 characterised in that one or more of the substitutions is selected from the group consisting of K-16, K-185, K-11, K-44, K-210, R-63, K-13, F-6, K-149, K-128, E-184, K-112, F-157, E-3, K-29, N-203, N-34, K-78, K-151, L-15, L-158, Y-102, W-186, K-134, D-87, K-52, T-67, T-125, K-150, Y-40, Q-48, L-65, K-81, Q-101, Q-208, K-144, N-8, N-70, H-104, Q-45, K-137, K-159, E-205, N-82, A-111, D-131, K-24, --V-36, N-7, M-138, T-209, V-84, K-172, V-19, D-56, P-73, G-33, T-106, N-170, L-28, T-43, Q-114, C-10, K-60, N-31, K-47, E-5, D-145, V-38, A-127, D-156, E-204, P-71, G-26, Y-129, D-141, F-201, R-68, N-200, D-49, S-153, K-35, S-39, Y-25, V-37, G-18, W-85 and I-182.

35. A recombinant allergen according to any of claims 1-34 for use as a pharmaceutical.

36. Use of the recombinant allergen according to any of claims 1-34 for preparing a pharmaceutical for preventing and/or treating allergy.

37. A composition comprising two or more recombinant mutant allergen variants according to any of claims 1-34, wherein each variant is defined by having at least one primary mutation, which is absent in at least one of the other variants, wherein for each variant no secondary mutation is present within a radius of 15 Å from each

absent primary mutation.

38. A composition according to claim 37 comprising 2-12,
preferably 3-10, more preferably 4-8 and most preferably
5 5-7 variants.

39. A composition according to claim 37 or 38 for use as
a pharmaceutical.

10 40. Use of a composition according to claim 37 or 38 for
preparing a pharmaceutical for preventing and/or treating
allergy.

41. A pharmaceutical composition, characterised in that
15 it comprises a recombinant allergen according to any one
of claims 1-34 or a composition according to claim 37 or
38, optionally in combination with a pharmaceutically
acceptable carrier and/or excipient, and optionally an
adjuvant.

20 42. A pharmaceutical composition according to claim 41,
characterised in that it is in the form of a vaccine
against allergic reactions elicited by a naturally
occurring allergen in patients suffering from allergy.

25 43. A method of generating an immune response in a
subject comprising administering to the subject a
recombinant allergen according to any one of claims 1-34,
a composition according to claim 37 or 38 or a
30 pharmaceutical composition according to claims 41 or 42.

44. Vaccination or treatment of a subject comprising
administering to the subject a recombinant allergen
according to any one of claims 1-34, a composition
35 according to claim 37 or 38 or a pharmaceutical
composition according to claims 41 or 42.

45. A process for preparing a pharmaceutical composition according to claim 41 or 42 comprising mixing a recombinant allergen according to any one of claims 1-34
5 or a composition according to claim 37 or 38 with pharmaceutically acceptable substances and/or excipients.

46. A pharmaceutical composition obtainable by the process according to claim 45.

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47. A method for the treatment, prevention or alleviation of allergic reactions in a subject comprising administering to a subject a recombinant allergen according to any one of claims 1-34, a composition
15 according to claim 37 or 38 or a pharmaceutical composition according to any one of claims 41-42 or 46.

48. A method of preparing a recombinant allergen according to any one of claims 1-34, characterised in

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- a) identifying a number of amino acid residues in a naturally occurring allergen, which has a solvent accessibility of at least 20 %;
- 25 b) selecting at least four of the identified amino acid residues in such a manner that each selected amino acid is spaced from each other selected amino acid by at least 15 Å, and that the selected amino acids are placed in such a manner that at least one circular surface region
30 with a area of 800 Å² comprises no selected amino acid; and
- c) effecting for each of the selected amino acids a primary mutation, which reduce the specific IgE binding
35 capability of the mutated allergen as compared to the binding capability of the said naturally occurring

allergen, wherein each primary mutation is a substitution of a selected amino acid residue with another amino acid, which does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic species from which said naturally occurring allergen originates.

49. A method according to claim 48, characterised in ranking the said identified amino acid residues with respect to solvent accessibility and substituting one or more amino acids among the more solvent accessible ones.

50. A method according to claim 48 or 49, characterised in selecting identified amino acid residues, which are conserved with more than 70 % identity in all known homologous proteins within the species from which said naturally occurring allergen originates.

51. A method according to claim 50, characterised in ranking the said identified amino acid residues with respect to degree of conservation in all known homologous proteins within the species from which said naturally occurring allergen originates and substituting one or more amino acids among the more conserved ones.

52. A method according to any of claims 48-51 comprising selecting the identified amino acids so as to form a mutant allergen, which has essentially the same α -carbon backbone tertiary structure as said naturally occurring allergen.

53. A method according to any of claims 48-52 characterised in that the substitution of amino acid residues is carried out by site-directed mutagenesis.

54. A method of preparing a recombinant allergen

according to any one of claims 1-34, characterised in that the allergen is produced from a DNA sequence obtained by DNA shuffling (molecular breeding) of the DNA encoding the corresponding naturally occurring.

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55. A DNA sequence encoding a recombinant allergen according to any of claims 1-34, a derivative thereof, a partial sequence thereof, a degenerated sequence thereof or a sequence, which hybridises thereto under stringent
10 conditions, wherein said derivative, partial sequence, degenerated sequence or hybridising sequence encodes a peptide having at least one B cell epitope.

56. A DNA sequence according to claim 55, which is a
15 derivative of the DNA sequence encoding the naturally occurring allergen.

57. A DNA sequence according to claim 56, wherein the
20 derivative is obtained by site-directed mutagenesis of the DNA encoding the naturally occurring allergen.

58. A DNA sequence according to any of claims 55-57, wherein the sequence is a derivative of the sequence shown in Fig. 3, wherein the DNA sequence is mutated so
25 as to encode an allergen having at least four mutations selected from the group consisting of V2, D72, E87, K-129, E-60, N-47, K-65, P-108, N-159, D-93, K-123, K-32, D-125, R-145, D-109, E-127, Q-36, E-131, L-152, E-6, E-96, D-156, P-63, H-76, E-8, K-134, E-45, T-10, V-12, K-
30 20, S-155, H-126, P-50, N-78, K-119, V-2, L-24, E-42, N-4, A-153, I-44, E-138, G-61, A-130, R-70, N-28, P-35, S-149, K-103, Y-150, H-154, N-43, A-106, K-115, P-14, Y-5, K-137, E-141, E-87 and E-73.

35 59. A DNA sequence according to any of claims 55-57, wherein the sequence is a derivative of the sequence

shown in Fig. 13, wherein the DNA sequence is mutated so as to encode an allergen having at least four mutations selected from the group consisting of K-16, K-185, K-11, K-44, K-210, R-63, K-13, F-6, K-149, K-128, E-184, K-112, F-157, E-3, K-29, N-203, N-34, K-78, K-151, L-15, L-158, Y-102, W-186, K-134, D-87, K-52, T-67, T-125, K-150, Y-40, Q-48, L-65, K-81, Q-101, Q-208, K-144, N-8, N-70, H-104, Q-45, K-137, K-159, E-205, N-82, A-111, D-131, K-24, V-36, N-7, M-138, T-209, V-84, K-172, V-19, D-56, P-73, G-33, T-106, N-170, L-28, T-43, Q-114, C-10, K-60, N-31, K-47, E-5, D-145, V-38, A-127, D-156, E-204, P-71, G-26, Y-129, D-141, F-201, R-68, N-200, D-49, S-153, K-35, S-39, Y-25, V-37, G-18, W-85 and I-182.

60. A DNA sequence according to any of claims 55-57, wherein the sequence is a derivative of the sequence shown in Fig. 16, wherein the DNA sequence is mutated so as to encode an allergen having at least four mutations selected from the group consisting of R-128, D-129, H-11, H-30, S-1, K-77, Y-75, R-31, K-82, K-6, K-96, K-48, K-55, K-89, Q-85, W-92, I-97, H-22, V-65, S-24, H-74, K-126, L-61, P-26, N-93, D-64, I-28, K-14, K-100, E-62, I-127, E-102, E-25, P-66, L-17, G-60, P-95, E-53, V-81, K-51, N-103, Q-2, N-46, E-42, T-91, D-87, N-10, M-111, C-8, H-124, I-68, P-79, K-109 and R-128, D-129, H-11, H-30, S-1, K-77, Y-75, R-31, K-82, K-6, K-96, K-48, K-55, K-89, Q-85, W-92, I-97, H-22, V-65, S-24, H-74, K-126, L-61, P-26, N-93, D-64, I-28, K-14, K-100, E-62, I-127, E-102, E-25, P-66, L-17, G-60, P-95, E-53, V-81, K-51, N-103, Q-2, N-46, E-42, T-91, D-87, N-10, M-111, C-8, H-124, I-68, P-79, K-109 and K-15.

61. An expression vector comprising the DNA according to any of claims 55-60.

62. A host cell comprising the expression vector of claim

63. A method of producing a recombinant mutant allergen comprising the step of cultivating the host cell
5 according to claim 62.

65. A diagnostic assay for assessing relevance, safety or
outcome of therapy of a subject using a recombinant
15 mutant allergen according to any of claims 1-34 or a
composition according to claim 37 or 38, wherein an IgE
containing sample of the subject is mixed with said
mutant or said composition and assessed for the level of
reactivity between the IgE in said sample and said
20 mutant.